

Electrophoresis: What does a century old technology hold for the future of separation science?

Khee Chian Jason Chin¹, Kah Hay Yuen², Chin Chin Siew³ and Beow Chin Yiap^{4*}

1. School of Postgraduate Studies & Research, Faculty of Medicine and Health, International Medical University, No. 126, Jalan 19/155B, Bukit Jalil, 57000 Kuala Lumpur, Malaysia
2. School of Pharmaceutical Science, Universiti Sains Malaysia, Minden, 11800 Penang, Malaysia.
3. Department of Microbiology, Faculty of Biotechnology and Biomelecular Sciences, Universiti Putra Malaysia, 43400 UPM SERDANG, Selangor, Malaysia.
4. Department of Life Sciences, School of Pharmacy and Health, Faculty of Medicine and Health, International Medical University, No. 126, Jalan 19/155B, Bukit Jalil, 57000 Kuala Lumpur, Malaysia.

* **Corresponding author.** Beow Chin Yiap

ABSTRACT: Electrophoretic separation was first demonstrated in the year of 1807 and has since been a staple tool used by biologists and chemists for more than a century since its inception. From the initial crude paper electrophoresis system to today's modern automated electrophoresis system, the development of electrophoresis systems have been driven by the advancement of technology such as miniaturization, precision engineering, biochemistry, electrical and electronics. These advancements were introduced to meet the requirement for faster and better resolution of results. This paper reviews the evolution of the electrophoresis technology over one century and provides an insight into the possible future development of electrophoresis. Various aspects of the electrophoresis system such as the performances, designs, usages, separation phases, and biochemistry were analysed. The technological advancements for this field have been evidenced by the increasing complexity of the electrophoresis system. A peek into the possible future for the world of electrophoresis has been provided by drawing insights from the missing links of current technologies. It is both exciting and equally perplexing to explore the promises that this seeming simple separation technology holds for the future.

Keywords: Development; Electrophoresis; Future; History; Progress;

INTRODUCTION

Historical development of electrophoresis

The concept of electrophoresis was discovered a couple of centuries ago, in 1807 by Ruesch [1] who conducted an experiment of applying an electrical current through a suspension of clay in water. He noted the migration of particles towards the anode. However, it was not until the year 1942, when Coleman and Miller [2], conducted an experiment and discovered the migration of neutral hexose toward the anode with a borax (sodium borate) solution that electrophoresis became a major scientific method. Many different type of variable experiments was carried out to determine the usage and limitations for the electrophoresis of other compounds, such as those containing adjacent “-OH” groups and also high concentration of neutral sugars [3, 4, 5]. After a successful separation of sugar was conducted in 1952 by Consden and Stanier [6], electrophoresis was then used for DNA and RNA separation. Around the 1970's, advancements in the process of electrophoresis in the area of DNA and RNA separation flourished, with most of today's current technology in electrophoresis emerging from then and is still being used until the present time.

Some of the notable experiments conducted during the 1970's were by Richards *et. al.* [7] who used a Tris-based media to separate RNA, introducing the use of Tris media, Tris-acetate-EDTA (TAE) and Tris-borate-EDTA (TBE), as the buffer solution for subsequent studies. Another experiment which defined the usage of electrophoresis as a tool for analysis of DNA was conducted by Danna *et. al.* [8] in which they introduced the usage of restriction enzyme, produced by *Hemophilus inXuenzae*, to determine the relative molarity and length of fragments of SV40 DNA. The use of ethidium bromide as a DNA stain was introduced when it was used in the conductive medium to show the differences between linear and circular DNA by Aaij and Borst in 1972 [9].

For the conductive buffer (running buffer), Tris emerged as the dominating buffer to be used. There are no known reasons behind Tris being the dominant buffer as there have been many alternative chemicals. Tris-borate-

EDTA (TBE) was introduced at 1968 by Peacock, *et. al.*, [10] and Tris acetate acid EDTA (TAE) was introduced at 1972 by two separate groups [8, 9]

In the past for DNA analysis work to be conducted to determine its size, a lengthy procedure must be conducted [11]. Electrophoresis uses the electrical field to separate and move molecules through a sieve like compound based on the molecular mass and charge ratio [12]. Electrophoresis is usually used for molecular biology work (ie DNA, RNA and protein separation). The process of electrophoresis can also be used for the analysis of chemistry compound such as water, soil and air quality or contamination, food quality and processing hygiene and also for possible medical forensic analysis. The fundamental principle of electrophoresis is applied for these processes with only a different type of detection system being used [13]. DNA, RNA, and protein works is not only limited to just separation. Electrophoresis can also be used as a purification process. This is usually done in cases where the research is limited to specific targets of interest, for example a membrane protein or a specific gene. In this situation, electrophoresis is used to separate and the protein or gene of interest is excised out from the entire sample [13].

Types and advancement of Electrophoresis

Since the introduction of electrophoresis into the scientific world, many changes and improvements to the resolution, resolving, and efficiency abilities has been made to the system. From the basic paper electrophoresis to the highly advance automated microchip electrophoresis system, each different system has its own functionality and unique usage.

Paper electrophoresis

Paper electrophoresis is one of the simplest techniques of electrophoresis. In this technique the sample is applied onto a point of a strip of filter paper that has been moisturized with a buffer solution. Each end of the strip is then dipped into separate tanks containing the buffer solution and a different electrode (anode or cathode). An electric current is then applied and the sample will then move towards the electrode with the opposite polarity. When the process is done, the strip is then dried and viewed with a detection system [14].

This technique has often been compared to paper chromatography due to their similarity in their mode of action. However, the difference between these two systems is that chromatography separates a sample based on its polarity while electrophoresis separates the sample based on its charge by applying a running electrical charge from one terminal to the other [14].

Agarose gel electrophoresis

Agarose is a polysaccharide that is able to form pores with sizes ranging from 100 to 300 nm in diameter, with the size of the pore correlating with the concentration of the agarose gel. The higher the concentration of the agarose gel the smaller the pore size.

Agarose gel electrophoresis is often used to separate DNA or RNA fragments of different length. This technique involved the movement of charged molecule in an electric field, in which a positive and negative electrode (i.e. anode and cathode), will allow the negatively charged DNA or RNA molecules to migrate from the negative electrode to the positive electrode. The molecules are separated based on their length, size and structure [15].

Polyacrylamide gel electrophoresis

There are two types of gel; dissociating and non-dissociating gel. A non-dissociating gel separates the proteins in their native form, which helps to conserve the protein structures, functions and activity. It is useful when the protein of interest is wanted at the end of the procedure for experiment. A dissociating gel denatures the protein into its constituent polypeptides. It is commonly used to determine the polypeptide composition of the sample [14].

Native gel electrophoresis is a non-denaturing gel that has a higher resolving power than the SDS-PAGE. It is also used for protein separations. However, it does not denature the proteins, unlike SDS-PAGE which does [15].

Polyacrylamide gel electrophoresis consist of polyacrylamide gel, which is made up of chains of acrylamide monomers ($\text{CH}_2=\text{CH}-\text{CO}-\text{NH}_2$) that is cross linked with N, N'-methylenebisacrylamide units ($\text{CH}_2=\text{CH}-\text{CO}-\text{NH}-\text{CH}_2-\text{NH}-\text{CO}-\text{CH}-\text{CH}_2$), also commonly known as a "bis". The pore size of the polyacrylamide gel is also determined by the concentration of "bis". Polyacrylamide gel is used to separate strands of DNA molecules whose size is very close to each other, often by one nucleotide such as in SNP, and also proteins. This is because the polyacrylamide gel has a much higher resolving power than agarose gel electrophoresis [16].

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is a low cost, reproducible and rapid method for quantifying, comparing and also to characterize protein, peptides and small molecular weight nucleic acids [17]. This technique of electrophoresis separates proteins by their molecular weight [18].

There are two variant of the SDS-PAGE technique, the gradient and SDS-Urea gels. Both are based on the SDS-PAGE electrophoresis but with a higher resolving power based on the needs of the sample. Gradient gels are often used to separate small and large proteins in a single gel. SDS-Urea gels are used when the charge of the proteins is significantly relative to the mass of the protein, such as membrane proteins and immunoprecipitates as SDS-urea gel helps to solubilizes, denatures and dissociates the polypeptides chains without altering the proteins intrinsic charge, allowing the proteins to be separated base on their net charge [15].

Denaturing Gradient Gel Electrophoresis (DGGE) and Temperature Gradient Gel Electrophoresis (TGGE)

Denaturing Gradient Gel Electrophoresis (DGGE) is a method that is employed to separate PCR-generated DNA products for molecular fingerprinting. DGGE is different from conventional agarose gel electrophoresis as it separates the PCR products based on its sequence size differences and also its rate of denaturing. This specialty makes it important as conventional agarose gel electrophoresis only separates based on size only, which makes it improbable for molecular fingerprinting as some samples may have PCR fragments of similar size, thus forming only a single band or bands that are only slightly distanced apart it is impossible to determine [19].

As the samples pass through the gradient denaturing gel, it slowly denatures as the concentration of chemical denaturant in the polyacrylamide gel. When the sample reaches a threshold denaturant concentration, PCR samples with weaker bonds will denature much quickly, thus its migration along the polyacrylamide matrix will slow down dramatically, forming a more diverse set of bands which can then be easily compared to references online [19].

The other type of gel is TGGE, which functions principally similar to those of DGGE, except it uses a temperature gradient as its separating function. In TGGE, the gradient temperature is introduced perpendicularly from the sample running directions. This allows the sample to migrate from the low temperature to the high temperature, which allows the sample to denature accordingly based on site mutations. TGGE enables researchers to distinguish single base pair mutation in a single sample as the denaturing point could be either higher or lower from the original sample [20].

Isoelectric focusing and 2-dimension (2D) gel electrophoresis

Isoelectric focusing is a technique of separating the proteins based on their net charge, or also known as the isoelectric point of the protein. This is done by placing the sample of proteins in a pH gradient slab that is generated by an electric field. This will cause the proteins to migrate in the pH gradient field until it reaches a pH in which its isoelectric point is zero [21, 22].

For 2D gel electrophoresis, the system utilizes the SDS-PAGE and isoelectric focusing techniques. The technique of 2D gel electrophoresis separates the proteins based on their size and also their isoelectric point, thus giving a much better resolution of the protein and can also be used to separate a protein if the charge and size of the protein is known.

The advantage of the higher resolving power of the isoelectric focusing and 2D electrophoresis techniques allows for rapid and accurate analysis of proteins. However, with such high sensitivity there is a higher chance of getting errors. These techniques are highly sensitive to charge differences, and as such the samples needed to be handled with care, as any interactions of the sample with other lipids or other proteins may cause a change in the charge of the protein of interest, thus getting a negative result [22].

Zymography

Zymography is an electrophoresis technique which allows enzyme activity to be analysed *in situ* after the process of electrophoresis has been conducted on the enzyme [23]. This technique is important as it allows the researcher to characterize the protein or enzyme on the gel without the need for the researcher to purify the protein or enzyme of interest via excising the band from the gel and "cleaning" or extracting the band from the gel before any test could be conducted on it [24]. The use of zymography allows researchers to save time by removing the need to conduct such steps for protein or enzyme characterization.

There are many ways for zymography staining to be conducted. A paper by Coughlan [24] explains some of the methods used such as immersing the gel in a chemical reagent which highlights the bands on the gel. Another method mentioned is the use of second gel which is run concurrently with the gel containing the samples,

in which the second gel contains auxiliary enzymes and chromogenic reagents which allow the bands of interest to be detected on the second gel and allows for immunological detection [24].

One of the advantages of zymography over conventional assays is that it allows the enzyme activity to be studied based on their physical characteristics such as molecular weight or isoelectric point [23]. As the examination of the sample is conducted on the gel, many other factors can be studied such as posttranslational modification of a particular enzyme, heterogeneity of enzyme isoforms and studying enzyme activity in their native state, in which such studies cannot be conducted in standard conventional assays [23].

The application of zymography can vary from analyzing of ribonucleases using normal electrophoresis or isoelectric focusing, two-dimensional analysis of bacteria, fungal works, and also for detection and characterization of microbial proteases [25, 26, 27, and 28]. Zymography is also used as a way to double check on result, which enables researchers to check on suspected protein-protein or enzyme interaction [29]

Pulsed-field electrophoresis

For very large DNA molecules around 30 to 50kb, it is not capable to be separated using normal electrophoresis process [30]. This is because as the large DNA molecule migrates along the gel using normal electrophoresis, one end of the molecule will penetrate the matrix while the rest of the molecule trails along, forming a “snake” like smear [30]. Pulsed-field electrophoresis was therefore created to counter this situation. In this process, the electrical voltage periodically switches between three directions, one that runs through the central axis of the gel and two that run at an angle of 120° on each side of the gel. With this, the large DNA molecule is allowed to re-orientate, thus preventing the formation of the “snake” like smear. However, the larger the molecule the longer it will take to re-orient [30].

Capillary electrophoresis

Capillary electrophoresis is carried out in very thin capillary tubes, with about 1 to 10 µm inner diameter, that is usually made out of glass, quartz or plastic. The tube is then filled with any of the required buffer such as Laemmli for proteins or TAE buffer for agarose related work. A capillary electrophoresis run is very short, thus it is very useful for analytical work. Also, each reaction only takes a small amount of materials due to the small size of the capillary tubes. Capillary electrophoresis is suitable for use in genetic analysis, pharmaceuticals with enantiomers, counter-ion analysis in drug discovery, and protein characterization [14].

Many types of capillary electrophoresis systems exist. Some examples of are the micellar electrokinetic capillary chromatography, capillary electrochromatography, and the capillary array electrophoresis. Capillary array electrophoresis is an automated system that is often used for large scale projects, such as the Human Genome Project, as it allows massive screening of samples simultaneously [31]. Micellar electrokinetic capillary chromatography (MEKC) is used when the sample being analyzed contains a mixture of natural organic substance with similar chemical structure [32]. An ionic surfactant is mixed into the background electrolyte with a concentration higher than the critical micelle concentration. This allows the formation of charged micelles, which allows for the sample to migrate at a pseudostationary phase in the aqueous phase causing the separation to separate differently in the run [32]. Capillary electrochromatography (CEC) is alike to liquid chromatography, except it uses an electrical field to propel the mobile phase through the stationary phase [33, 34]. This allows it to have a higher efficiency capillary zone electrophoresis and high performance liquid chromatography. The application uses a capillary with a very small diameter that is packed with a solid stationary phase, in which it allows for the electrical field to move the mobile phase through it, reduced plate heights as a result of the plug flow profile and the ability to use smaller particles leading to higher peak efficiency than is possible in pressure driven systems [33, 34].

There are numerous ways to detect and accumulate the data from the samples. One example of detection systems used for capillary electrophoresis is laser induced fluorescent; the detection limit is increase, as it is able to detect smaller concentration of sample but it is a much harder and more expensive detection system to use [14]. Another method used is by the electrically floating conductivity detection system. This system is designed to be separated from the main capillary electrophoresis body. The detection system detects the conductivity by measuring the electrodes. The electrical signal that is generated by the electrodes will pass through the floating electronics and transmitted via infrared to a computer as data [35]. An experiment conducted by Tristezza *et. al* [36] details protocol and usage of capillary electrophoresis for the identification of protein markers in *Saccharomyces cerevisiae*. In the paper, the result from using capillary electrophoresis was compared against agarose electrophoresis to evaluate their differences in detection limit [36]. Another advantage of using capillary electrophoresis is that due to its high precision and reading capabilities, it is used for estimation of unknown DNA or RNA fragment size [37].

Microchip electrophoresis

A further advancement to the capillary electrophoresis system, the microchip electrophoresis system boasts a more efficient system. One of the main benefits is an increase in throughput by many folds over the capillary electrophoresis system as the microchip systems contains numerous microchannels which allow high throughput experiments to be conducted quickly and efficiently [38].

Another benefit is the low fabrication cost as the intricate enclosed microchannels is comprised of glass or fused silica substrates. These materials enable ultra-fast DNA separations as the sample loading format is unique, coupled with short separation distances and optimal thermal characteristics of the glass or fused silica substrates. The microchip electrophoresis system is also fully automated, from sample handling to data analysis, which allows minimal human handling and possible error [38]. Example of this system is the "lab on a chip" device.

Microchip electrophoresis uses laser-induced fluorescence and electrochemical detection method as their detection method due to the size of the microchip and as well as the required accuracy needed to read their results require highly accurate methods for microchip electrophoresis to work [39, 40].

Research in developing new materials for microchip electrophoresis is widely delved into by many groups of researchers [41, 42, 43, 44]. Many different type of materials have been used to fabricate the microchip, from the most common material such as silica and glass substrates, to poly(dimethylsiloxane), or PDMS, and poly(methylmethacrylate), or PMMA, as a material to fabricate microchip electrophoresis via thin-casting method [41, 42]. The advantage of this protocol reduces the cost of production and also allows faster results development. Wang *et. al.* [42] also proposed new materials such as silver ink to be used as electrode for microchip electrophoresis. The materials that are normally used as electrodes are platinum or platinum-iridium alloy, gold, copper and aluminium [43, 44].

Fluorophore-assisted carbohydrate electrophoresis (FACE)

FACE is used to identify carbohydrates with an attached fluorescent dye by separating the carbohydrates using a polyacrylamide gel [44]. This technique is important as carbohydrates are not charged, and is the main technique used to analyze different types of carbohydrates such as plant and bacterial polysaccharide [44]. Another advantage to using FACE is to reduce the need for complex work such as those conducted by Gao [45]. For that project, FACE enables the researchers to simplify a process to detect lipid-linked oligosaccharides, which would require the samples to be metabolically labeled with radioactive sugar precursors before the molecule of interest can be detected [46].

Affinity electrophoresis

Affinity electrophoresis systems is a technique in which the resolving capability of capillary electrophoresis is used to separate samples that undergoes specific or non-specific affinity interactions during the process of electrophoresis [47]. This process can occur in either solution or immobilised to a solid support [48]. It is also used to measure the binding affinity of receptors to neutral and charged ligands [49]. The use of affinity electrophoresis is extensive in that it is able to detect affinity interactions in either free or immobilised form. Some of its uses include detection for peptides and proteins, drugs development, detection of small molecules and also for immuno-affinity works [50-59]. There are also different types of affinity systems. For example, Taketa and Hirai [60] explain the usage of lectin affinity for use on serum α -fetoprotein to determine the interaction between lectin and ligand. Smith and Kelleher [61] used concanavalin A affinity chromatography as an alternative detector module.

Some of the advantages of using affinity electrophoresis is that the sensitivity of the technique allows for more precise detection and discrimination of normal and carcinogenic proteins from the same sample [62]. Also, its wide field of usage makes it an important technique for sampling and data collection.

Automated electrophoresis system

With the advancement of technology, it is now possible to conduct electrophoresis by using computerized robotics and programming that enables electrophoresis protocols to be conducted automatically. Automated systems come in many different types and form. With today's technological advances, various types of electrophoresis systems can be automated. For example, Michels *et. al.*[63] describe the works of an automated 2D capillary electrophoresis system which they used for high throughput protein analysis. Another work conducted by Kristensen *et. al.*[64] shows the usage of various automated systems, including an automated electrophoresis system, for the Human Genome Project to detect variation in the human gene. Automated electrophoresis systems are also highly accurate and precise and can even detect single-strand conformation polymorphism in genetic samples [65]

One of the advance automated system is the automated buffer-less electrophoresis system which uses pre-casted gel, either SDS or agarose, that does not require any buffer to run, which is one it biggest advantage. The system boast the possibility to view the progress of the gel run by attaching the machine to a ultra-violet light or blue light viewing add-on [66, 67]. It is capable of separating different types of sample by using the appropriate gel type at high speed.

Preview of future trends

With the development of increased sensitivity in detection systems, it is possible to increase the speed and processing of electrophoresis. This also enables researchers to use less of their samples, which is the best advantage of capillary electrophoresis, as it will mean less preparation time and also less wastage of sample.

Although the technology of electrophoresis has advanced tremendously from the basic paper electrophoresis system to today's highly advance microchip electrophoresis system, there is still a basic need for an external power supply to run the electrophoresis, more so as the system becomes more sophisticated. This serves as a bottle neck on the usage of any electrophoresis system in that the usage of any system is limited to a room with an external power supply. Without a power supply electrophoresis cannot be conducted. Active research is being carried out currently to overcome these limitations (Unpublished results).

Other disadvantages of the current electrophoresis systems and techniques were investigated by Chery, *et. al.* [68]. In the paper, the influence of contaminations such as vanadium and selenium can compromise gel results, especially if the process is being left to run for a long period of time. However, the authors stressed that more testing needed to be carried out regarding the effect of contaminations on gel results viability.

With capillary electrophoresis, a lot of care into the details, such as capillary position and gas flow rates, of each experiment runs have to be taken to be able to reproduce the same results as differences in the settings will generate different results for the same experiment [69].

With the advancement in technology, both in engineering and sciences, it is now possible to produce smaller circuit boards that is light and has higher efficiencies and more efficient transformers. Portable electrophoresis system could be built on these. Currently there is preliminary research into designing such a system but the efficiency of such systems is still being tested. For example, Zhang *et. al.* [70] described their method to design a portable capillary electrophoresis with a conductivity detector. They discussed the use of microchip technology coupled with integrated electrodes which would act as a detector to transmit the data to be analyzed by a separate machine to generate its result. Another research project that takes advantage of the increasing technology development has developed a method in which the staining and de-staining of nucleic acid to be automated and is cheap [71]. The paper describes the method, in which the researchers use day to day materials such as a model airplane fuel pump to circulate the staining reagents via the reagent bottles to the staining chamber, which cuts cost for low budget laboratory when compared to buying and using commercial systems [71].

Also, there are many patents regarding increasing the efficiencies of current systems, such as using a different buffer system or gel type, in increasing the efficiency. There is major research being done by different groups around the world on improving currently used systems, notably the automated electrophoresis system. The main point of the research being done is to increase the detection limit and also the reduction in the amount of background noise [72] Also, the uses of different type of materials, such as coated capillaries, for running gel electrophoresis and upgrading the detection system on the current equipments are also being looked into [17, 73, 74, 75, 76, 77, 78, 79, 80]. The materials being research and developed by these groups mainly focuses on increasing the efficiencies, reproducibility and accuracy of the separation run. Work is also being done to enable retrieval of the separated sample without damaging it [77].

CONCLUSION

The technology of electrophoresis started in the beginning of the nineteen century and even after two centuries have passed it still remain as relevant as it has been in the separation science. Although present electrophoresis is being done in many different ways and method that the equipment and style is so different from the original design, yet the core principle remains the same. By following the trends in changes to the technology of electrophoresis, the next step of development would be miniaturization and portability of systems.

Competing interest

The authors declare no competing interest.

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